Claims

1. A process for obtaining mammalian insulin secreting cells in vitro, characterized in that it contains the following steps:

a) preparation of the mammalian pancreatic tissues from previously removed pancreata,

b) dissociation of the pancreatic tissues obtained in step (a) into isolated pancreatic cells,

c) possibly the elimination of the endocrine cells from the pancreatic cells isolated in step (b),

d) induction of dedifferentiation of the cells isolated in step (b) into ductal precursor cells,

e) induction of redifferentiation of the ductal precursor cells obtained in step (d) into insulin secreting cells.

2. A process according to Claim 1, characterized in that the dissociation of the pancreatic tissues in step (b) is carried out by enzymatic digestion.

3. A process according to either of Claims 1 and 2, characterized in that the elimination of endocrine cells in step (c) is carried out by means of density gradient centrifugation.

4. A process according to any one of Claims 1 to 3, characterized in that the elimination of the endocrine cells is carried out by withdrawal of the fraction of the endocrine cells recovered in a density range between 1.027 g/L to 1.104 g/L, preferably between 1.045 g/L to 1.097 g/L.

5. A process according to any one of Claims 1 to 4, characterized in that the exocrine cells devoid of endocrine cells are recovered after centrifugation of the pancreatic cells isolated in step (b), in the pellet from the density gradient residue.

6. A process according to either of Claims 1 or 2, characterized in that the elimination of the endocrine cells is carried out by means of a cell separator.

7. A process according to any one of Claims 1 to 6, characterized in that the dedifferentiation of step (d) includes the following substeps:

i) culturing of the cells obtained in step (c) with a cell concentration between 1 x 10^6 and 10 x 10^6 cells/mL, preferably between 2 x 10^6 and 6 x 10^6 cells/mL, in a culture medium containing:

-glucose at a concentration between 1 and 10 g/L, preferably between 2 and 5 g/L.

-possibly serum, chosen from fetal calf serum, bovine serum or human serum, at concentrations greater than 8%, preferably between 10 and 15% final volume.

-a mixture of insulin, transferrin, selenium used at a concentration between 0.2 and 3%, preferably between 1.0 and 2.5%,

-possibly factors preventing the growth of fibroblasts at a concentration between 20 and 100 µg/mL, preferably between 30 and 60 µg/mL,

-possibly antibiotics, antifungal agents,

for a duration between 4 to 9 days, preferably 5 to 7 days,

ii) recovery of the ductal precursor cells obtained in step (i).

8. A process according to one of Claims 1 to 7, characterized in that the induction of the redifferentiation of step (e) includes the following substeps:

i) possibly the separation of the ductal precursor cells obtained in step (d)

ii) culturing of the ductal precursor cells obtained in step (i) at cell concentrations between 3.5 x 10^5 cells/25 cm^2 and 4 x 10^6 cells/25 cm^2, preferably 7 x 10^5 cells/25 cm^2 to 3 x 10^6 cells/25 cm^2, in a culture medium containing:

-glucose at concentrations between 1 and 10 g/L, preferably between 2 and 5 g/L.

-possibly serum, chosen from fetal calf serum, bovine serum or human serum, at concentrations greater than 2.5%, preferably between 5 and 15% final volume.

-possibly a mixture of insulin, transferrin, selenium at a concentration between 0.2 and 5%, preferably between 0.5 and 2%,

-possibly antibiotics and antifungal agents,

-possibly in the presence of a matrix,

for a duration between 12 and 36 h,

iii) withdrawal of said culture medium, and of the non-adherent cells possibly present,

iv) culturing of the cells obtained in step (iii) in a culture medium such as that used in step (i), possibly containing growth factors,

for a duration between 4 and 12 days, preferably between 5 and 10 days,

in order to obtain insulin secreting endocrine cells, and

v) recovery of the insulin secreting cells obtained in step (iv).

9. A process according to any one of Claims 1 to 8, characterized in that the separation of the ductal precursor cells obtained in substep (i) of step (e) is done with trypsin/EDTA at concentrations between 0.01 and 0.1% trypsin, preferably 0.015-0.03, and EDTA, between 0.1 and 1 mM, preferably 0.25-0.75 mM.

10. A process according to any one of Claims 1 to 9, characterized in that the matrix used for culturing of the cells in substep (ii) of step (e) is chosen from collagen type IV, 804G, collagen type I, Matrigel.

11. A process according to any one of Claims 1 to 10, characterized in that the pancreatic tissues prepared in step (a) were obtained from a previous removal of a fragment of the pancreas of a brain dead adult human.

12. A process according to any one of Claims 1 to 10, characterized in that the pancreatic tissues prepared in step (a) were obtained from a previous removal of a fragment of a pancreas of a living patient suffering from a pancreatic pathology.

13. A process according to Claim 12, characterized in that the pancreatic tissues prepared in step (a) were obtained from a previous removal of a fragment of a pancreas of a living patient suffering from diabetes.

14. A cell preparation which can be obtained by the process according to any one of Claims 1 to 13, characterized in that it has a cell concentration between 1×10^6 and 10×10^6 cells/mL, preferably between 2×10^6 and 6×10^6 cells/mL.

15. Use of a cell preparation according to Claim 14 for the preparation of a pharmaceutical composition which can be used for the treatment of pancreatic pathologies.

16. Use according to Claim 15 for the treatment of diabetes.

17. A bioartificial pancreas, characterized in that it contains insulin secreting cells which can be obtained by the process according to any one of Claims 1 to 13, cultured in a matrix.

18. A bioartificial pancreas, characterized in that it contains insulin secreting cells which can be obtained by the process according to any one of Claims 1 to 13, cultured in a matrix.